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Carsten G.B. Grupstra
Institute for Biodiversity and Ecosystem Dynamics - Amsterdam

Rafel Coma
CSIC - Centro de Estudios Avanzados de Blanes (CEAB)

Marta Ribes
CSIC - Instituto de Ciencias del Mar (ICM)

Karine Posbic Leydet
Louisiana State University

John Everett Parkinson
Pennsylvania State University

See next page for additional authors

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NOTE

Evidence for coral range expansion accompanied by reduced diversity of *Symbiodinium* genotypes

Carsten G. B. Grupstra[1,2,3] · Rafel Coma[4] · Marta Ribes[2] · Karine Posbic Leydet[5,6] · John Everett Parkinson[7,8] · Kelly McDonald[9] · Marc Catllà[2,4] · Christian R. Voolstra[3] · Michael E. Hellberg[5] · Mary Alice Coffroth[10]

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Abstract Zooxanthellate corals are threatened by climate change but may be able to escape increasing temperatures by colonizing higher latitudes. To determine the effect of host range expansion on symbiont genetic diversity, we examined genetic variation among populations of *Symbiodinium psygmophilum* associated with *Oculina patagonica*, a range-expanding coral that acquires its symbionts through horizontal transmission. We optimized five microsatellite primer pairs for *S. psygmophilum* and tested them on *Oculina* spp. samples from the western North Atlantic and the Mediterranean. We then used them to compare symbiont genotype diversity between an Iberian core and an expansion front population of *O. patagonica*. Only one multilocus *S. psygmophilum* genotype was identified at the expansion front, and it was shared with the core population, which harbored seven multilocus genotypes. This pattern suggests that *O. patagonica* range expansion is accompanied by reduced symbiont genetic diversity, possibly due to limited dispersal of symbionts or local selection.

Keywords *Oculina patagonica* · *Symbiodinium psygmophilum* · Zooxanthellae · Microsatellites · Climate change · Symbiosis

Introduction

Tropical coral-reef communities are declining worldwide due to several threats, including rising sea surface temperatures associated with climate change (Wilkinson 2008). However, some corals may be able to escape warming waters by expanding their range to higher latitudes (Precht and Aronson 2004; but see Muir et al. 2015). The ability of some zooxanthellate coral hosts to associate with different *Symbiodinium* types, or even switch between them, may contribute to acclimatization of the coral

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✉ Carsten G. B. Grupstra
cgb.grupstra@gmail.com

[1] Institute for Biodiversity and Ecosystem Dynamics, Faculty of Science, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

[2] Institut de Ciències del Mar, ICM-CSIC, Passeig Marítim Barceloneta 37-49, 08003 Barcelona, Spain

[3] Red Sea Research Center, Division of Biological and Environmental Science and Engineering (BESE), 4700 King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

[4] Centre d'Estudis Avançats de Blanes, CEAB-CSIC, Accés Cala Sant Francesc 14, 17300 Girona, Spain

[5] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

[6] Biology Department, Syracuse University, Syracuse, NY, USA

[7] Department of Biology, Pennsylvania State University, State College, PA, USA

[8] Department of Integrative Biology, Oregon State University, Corvallis, OR, USA

[9] Department of Biological Sciences, University at Buffalo, Buffalo, NY 14260, USA

[10] Graduate Program in Evolution, Ecology and Behavior and Department of Geology, University at Buffalo, Buffalo, NY 14260, USA

holobiont to newly encountered or changing environmental conditions (e.g., Silverstein et al. 2015), because unique *Symbiodinium* types exhibit variable tolerance to different environmental regimes (e.g., temperature and light; Thornhill et al. 2008; Hume et al. 2016). Therefore, higher *Symbiodinium* diversity within coral populations, combined with the ability of hosts to associate with different symbiont types, is expected to result in increased resilience to environmental change (Baskett et al. 2009).

Oculina patagonica is a shallow-water (0–10 m; Serrano et al. 2013) stony coral that reproduces through broadcast spawning and acquires algal symbionts through horizontal transmission (Fine et al. 2001). *Symbiodinium psygmophilum* (ITS2 type B2, LaJeunesse et al. 2012; cp23S type B224, Santos et al. 2003a) is the primary symbiont associated with *O. patagonica* (Leydet and Hellberg 2016). This coral is rapidly colonizing northern waters along the Mediterranean coast of the Iberian Peninsula (Serrano et al. 2013), providing an excellent opportunity to examine the effect of zooxanthellate coral range expansion on *Symbiodinium* genetic diversity.

In this study, we first optimized five microsatellite primer sets for *S. psygmophilum*, then used them to compare *S. psygmophilum* genotypic variation between two distant populations of *O. patagonica*: one population from the demographic core area of its distribution along the Iberian coastline and one in the expansion front.

Methods

Primers for five microsatellite loci were developed or optimized to examine genotypic diversity of the algal symbiont associated with *Oculina, S. psygmophilum* LaJeunesse, Parkinson and Reimer, 2012. *Oculina* samples were obtained from the western North Atlantic and the Mediterranean Sea. Populations of *Oculina* in the western North Atlantic were originally described as several taxa based on morphology; however, recent genetic data indicate the presence of genetically distinct populations that do not reflect the current morphologically based species classifications (Eytan et al. 2009). For simplicity, we refer to these samples as *Oculina* spp. Mediterranean *Oculina* populations consist only of *O. patagonica* de Angelis, 1908, which is genetically distinct from the western Atlantic *Oculina* spp. populations (Leydet and Hellberg 2015). *Oculina* spp. samples were acquired by Leydet and Hellberg (2016) from North Carolina ($n = 5$), Daytona Beach, FL ($n = 5$), Cape Florida, FL ($n = 4$), and Panama City, FL ($n = 5$). *Oculina patagonica* samples were acquired by Leydet and Hellberg (2016) from Italy ($n = 2$), Greece ($n = 2$), Israel ($n = 2$), and Lebanon ($n = 2$). Additional fragments of *O. patagonica* were collected from two Iberian populations: Cabo Cope ($n = 20$; mean depth 1.5 m), a "core" Iberian population (Zibrowius and Ramos 1983); and Roca de l'Illot ($n = 20$; mean depth 1.8 m), a population from the expansion front (Serrano et al. 2013) approximately 380 km north of Cabo Cope (Fig. 1). DNA was extracted using the Qiagen DNeasy blood and tissue kit modified for extraction of *Symbiodinium* DNA by adding a bead-beating step to rupture symbiont cell walls (Coffroth et al. 1992).

The presence of *S. psygmophilum* in all samples was verified using cp23S markers (224 bp fragment size, Santos et al. 2003a). Genotypic diversity of *S. psygmophilum* was examined using microsatellite loci. A total of 41 primer sets were screened, of which 22 were designed from the *S. psygmophilum* transcriptome (Parkinson et al. 2016) using PRIMER3 (Koressaar and Remm 2007; Untergasser et al. 2012). Microsatellite repeats were identified using SciRoKo (Kofler et al. 2007). Other primers tested were previously developed for *Symbiodinium* ITS2 types B1 and B7 (Santos et al. 2003b; Pettay and LaJeunesse 2007; Andras et al. 2009). Optimization yielded five primer sets (Table 1) with 2–9 alleles per locus (Electronic supplementary material, ESM, Table S1). All forward primers had an M13 sequence (5′-CAC GAC GTT GTA AAA CGA CG-3′) at the 5′ end for attachment of IRdye (Li-Cor Biotechnology). PCR reagent concentrations and thermocycler conditions varied between primer sets (see ESM Table S1 for methods). Because the forward primer for locus B2Sym17 (B2Sym17F1: 5′-CGA TGG AGG CAT ACA AGT GA-3′) failed to amplify some samples, an alternative forward primer was used (B2Sym17F2; Leydet and Hellberg 2016). Amplicons were visualized on

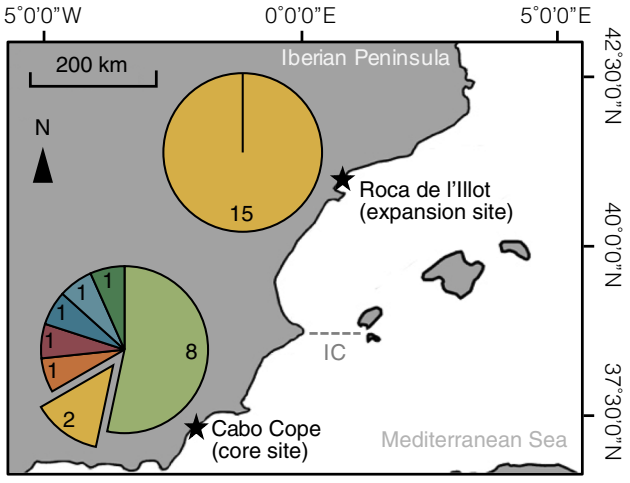

Fig. 1 *Symbiodinium psygmophilum* multilocus genotype diversity (MLG; based on four microsatellite loci) in a core (Cabo Cope) and expansion front (Roca de l'Illot) *Oculina patagonica* population. Unique MLGs are indicated in *different colors,* and the number of host colonies harboring a specific *S. psygmophilum* MLG is printed within. *IC* Ibiza Channel

polyacrylamide gels as described by Santos et al. (2003a). Fragments amplified using primer set B2Sym14 had variable sizes in the northern and eastern Mediterranean, but not at the two Iberian sites; therefore, this locus was not included during the assignment of multilocus genotypes (MLGs) for the latter two sites. Furthermore, Iberian samples that failed to amplify at one or several of the microsatellite loci were excluded (final $n = 30$, 15 per Iberian site). Linkage disequilibrium was tested at Cabo Cope to verify that the loci were independent (ESM Table S2). Lastly, remote sensing data for temperature, photosynthetically active radiation (PAR), and concentrations of chlorophyll *a*, phosphate and inorganic nitrogen were obtained for both Iberian study sites (ESM Table S3).

Results and discussion

Analysis of cp23S fragments verified that all samples contained *S. psygmophilum* (Santos et al. 2003a). The primer pairs reported here consistently amplified polymorphic alleles that differ within the western North Atlantic and the Mediterranean Sea, providing a useful tool for studying population differentiation in *S. psygmophilum*. All five microsatellite loci were polymorphic in at least one location (ESM Table S4). The western Atlantic host population harbored a diverse set of symbionts, reflecting Leydet and Hellberg (2016). Using our five microsatellite loci, we identified at least 12 MLGs across these populations ($n = 19$; Table 2). Multiple alleles were found at locus B2Sym17 in two *Oculina* spp. samples from Cape Florida, indicating the presence of multiple *Symbiodinium* genotypes within these colonies. No fragments were visualized for several samples screened with loci B2Sym14 and B2Sym17 (10 and 11, respectively) from Cape Florida, Daytona Beach and North Carolina. Direct sequencing using the B2Sym17 primers, however, resulted in detection of these alleles (Leydet and Hellberg 2016). We found seven unique MLGs within samples from the northern and eastern Mediterranean Sea (excluding Iberian sites; Table 2).

Within the Iberian sites, we identified seven unique MLGs at the core host population based on size variation at four microsatellite loci (B2Sym02, B2Sym04, B2Sym17 and Gv2_100). A single MLG was present in all *O. patagonica* individuals sampled at the expansion population and was shared with two individuals from the core population (Fig. 1). None of the microsatellite loci showed significant deviation from linkage equilibrium (ESM Table S2).

Several possible scenarios may explain reduced symbiont genotype diversity at the expansion front. First, because *O. patagonica* can propagate by means of polyp expulsion (Kramarsky-Winter et al. 1997), high rates of symbiont clonality within a coral population can be caused by asexual host propagation whereby host "offspring" maintain the "parental" symbiont genotypes. However, no *O. patagonica* clones were observed during a recent survey using the same samples from our expansion front study site, suggesting that asexual reproduction is not a major mechanism of propagation (Leydet 2016).

Since recruits acquire compatible *Symbiodinium* from the environment (Fine et al. 2001), migrating larvae or newly settled recruits in the expansion area must have acquired symbionts locally, i.e., at the expansion front. This suggests several not mutually exclusive possibilities: (1) the environmental pool has low *S. psygmophilum* diversity due to limited connectivity or a founder effect; (2)

Table 1 Sequences of forward (F) and reverse (R) primer sets for each microsatellite locus

Locus	Primer sequence (5′–3′)
B2Sym02	F: CTC CAT GTG CAA AGG GAA TC
	R: ATG GTG CTT TGT TCA GAC CC
B2Sym04	F: AGC CAA ACC GTG AAA CAA CT
	R: CCT GTC AGA GTT TGC CAC AA
B2Sym14	F: GCA CTC TAT CCA CAG CAC CA
	R: GCA TGA TAG GCA CTT GAG CA
B2Sym17	F2[a]: GCA ACA ATC ATA TTG ACT AGG CC
	R: GAT CCT ATT GGT GGT GGT GG
GV2_100[b]	F: CTA TCA AGG TCC TAT TTT CAC AGC ACA A
	R: A CAG GCG AGG TAT AGT ATT GAG TAA AAG AA

Reagent concentrations, thermocycler conditions, and motif sequences can be found in ESM Table S1. Size ranges per area are given in ESM Table S4

[a] Forward primer F2 was designed by Leydet and Hellberg (2016). The F1 primer sequence is presented in the text

[b] Gv2_100 forward and reverse primers were designed by Andras et al. (2009) for *Symbiodinium* B1

Table 2 Sample sizes (*n*) and number of *Symbiodinium psygmophilum* multilocus genotypes (MLGs) identified at each site based on four (Iberian study sites) or five (all other sites) microsatellite loci

Site	*n*	No. of MLGs
Panama City	5	3
Cape Florida	4	4
Daytona Beach	5	4
North Carolina	5	4
Greece	2	2
Israel	2	2
Lebanon	2	2
Italy	2	1
Cabo Cope	15	7
Roca de l'Illot	15	1

Genotypes were only shared between the core (Cabo Cope) and expansion (Roca de l'Illot) Iberian study sites

local selective pressures limit *Symbiodinium* diversity in the environmental pool; and/or (3) selection by the host and/or environment for specific symbiont pairings has resulted in local dominance of a single symbiont MLG.

Although symbiont species such as *S. psygmophilum* can have a broad distribution at the species level (LaJeunesse et al. 2012, 2014), dispersal of symbionts at the population level is generally thought to be limited (see review by Thornhill et al. 2017). Moreover, observations between 1992 and 2010 suggest that northward expansion of *O. patagonica* has occurred through a small number of long-distance dispersal events (between 76 and 182 km; Serrano et al. 2013), possibly limited by the oceanographic barrier represented by the Ibiza Channel (Fig. 1; Leydet 2016), and symbiont dispersal may have been similarly low. Subsequently, the most prevalent symbiont strains in the environment might be those that are released by nearby adult colonies (Nitschke et al. 2016), leading to dominance of a single symbiont MLG. However, two other cnidarians native to the expansion front (*Cladocora caespitosa* and *Bunodeopsis strumosa*) have been recorded to harbor *S. psygmophilum* in addition to clade A symbionts at several locations in the western Mediterranean Sea (reviewed by Casado-Amezúa et al. 2016), indicating that there are several putative sources of *Symbiodinium* at the expansion front. Therefore, environmental symbiont diversity here may not be shaped solely by limited dispersal rates.

Alternatively, given that unique genotypes within *S. psygmophilum* may exhibit functional variation (Parkinson et al. 2016), some of these genotypes might fare better under environmental conditions at the expansion front, potentially leading to limited diversity within the environmental pool (Pettay and LaJeunesse 2013; Baums et al. 2014). Similarly, the observed pattern of reduced symbiont genetic variation may be a result of predominant association of coral hosts with a distinct symbiont genotype well adapted to the prevailing local environment (e.g., Hume et al. 2016). Coral-associated symbiont distributions have been proposed to correlate with several environmental parameters including depth (LaJeunesse 2002; Kirk et al. 2009), PAR (Rowan et al. 1997) and temperature (Baums et al. 2014; LaJeunesse et al. 2014; Hume et al. 2016). While sampling depths were similar between the two populations, satellite data indicate that the expansion front site experienced lower yearly mean and minimum temperatures and lower mean PAR than the core population but higher concentrations of chlorophyll *a* and nutrients (phosphate, inorganic nitrogen; ESM Table S3). Low winter temperatures at the expansion site have been related to recurring patterns of partial colony mortality and subsequent tissue regeneration, which is energetically costly (Serrano et al. 2017). Therefore, local conditions may exert selective pressure on the environmental *Symbiodinium* pool and/or on host–symbiont associations.

In summary, our findings suggest that *O. patagonica* range expansion is accompanied by reduced genetic diversity of *Symbiodinium*, and this may be caused either by low diversity in the environmental pool, environmental selection of symbionts and/or selection for specific host–symbiont pairings. However, a more detailed genetic characterization of *S. psygmophilum* along the Iberian Peninsula using these new primer sets is needed to determine the most likely driver of the observed reduction in genetic diversity.

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